Neutralization

Immediate and complete neutralization of the active ingredient at all test time points is necessary in order to provide accurate evaluation of the test material

At the November 3, 1999 feedback meeting, the Agency requested additional information on the use of neutralizers in the first and all subsequent sampling steps of efficacy protocols. The Agency suggested that testing be done comparing the Industry proposed method with that used in New Drug Applications (NDA). Several NDAs for healthcare personnel handwashes containing chlorhexidine gluconate were retrieved through provisions of the Freedom of Information Act ("FOIA").

Although the methods contained similar elements such as neutralization after the final wash, and inoculum application, there is no one, consistent NDA test method. Consequently the Industry Coalition has generated data using the Health Care Personnel Handwash model comparing three different protocols based on the 1994 TFM proposal (FDA proposal), the 2000 ASTM protocol (Industry Coalition proposal) and the 1987 ASTM protocol (similar to many NDA submissions). We have also looked at the effect of neutralization on the efficacy of these products as they are reported in the literature. All the data indicate that immediate and complete neutralization of the active ingredient at all test time points is necessary in order to provide accurate evaluation of the test material.

The Industry Coalition is proposing the requirement of:

- Validation of the efficacy of the neutralizer used in any efficacy protocol;
- Immediate and complete neutralization in the first and all subsequent sampling fluids in all efficacy protocols.

Validation of the Efficacy of the Neutralizer used in any Efficacy Protocol

In antimicrobial efficacy testing, the number of bacteria surviving treatment is enumerated at specific sampling time points. Accurate determination requires effective neutralization of the antimicrobial ingredient at the specific sampling time points (Sutton, 1996). Inefficient or incomplete neutralization will permit killing of microorganisms to continue beyond the experimental exposure time, resulting in an over-expression of antimicrobial activity. This becomes especially critical when measuring antimicrobial efficacy over short (seconds to minutes) contact times when substantive active ingredients are used. Incomplete, or less than immediate, neutralization can introduce substantial errors in data.

Neutralization is key to ensuring the validity of the test. It is a key control point; therefore, the evaluation of the effectiveness of neutralization on antimicrobial ingredients cannot be ignored or understated. It acts as a control to the evaluation of the antimicrobial product. It is therefore necessary that the test parameters of the

antimicrobial effectiveness test be standardized in order to properly evaluate neutralization.

Two common neutralization methods employed in antimicrobial effectiveness tests are chemical inactivation and dilution. A few antimicrobial ingredients such as alcohols can be effectively neutralized by dilution; most, however, require the addition of chemical inactivator(s) to the dilution or sampling fluids to achieve neutralization.

There are four criteria to be met in designing a study of potential neutralizers (Sutton, 1996):

- The neutralizer must effectively inhibit the action of the antimicrobial formulation;
- The neutralizer itself must not be unduly toxic to the test organisms;
- The neutralizer and antimicrobial ingredient(s) must not combine to form a toxic compound; and
- The first three criteria must be demonstrated under conditions that mimic the actual conditions of the antimicrobial efficacy assay.

ASTM E 1054, *Standard Practices for Evaluating Inactivators of Antimicrobial Agents Used in Disinfectant, Sanitizer, Antiseptic, or Preserved Products*, or other suitable methods should be used when determining neutralizer effectiveness for *in vitro* and *in vivo* effectiveness tests of topical antiseptic drug products.

Neutralizer in the First and All Subsequent Sampling Fluids in all Efficacy Protocols

Two approaches were taken to evaluate the importance of neutralization in sampling procedures. First, a study comparing three handwash protocols was conducted using a known effective topical antimicrobial product that contained a substantive active ingredient. Second, an analysis of the literature data previously submitted in the August 6, 2001 Citizen Petition was conducted for the Healthcare Personnel Handwash, Surgical Scrub and Patient Pre-operative Preparation methods.

a. Healthcare Personnel Handwash Study - Protocol Comparisons

In discussions about the use of neutralizers in all sampling fluids, concern has been expressed about the effect of neutralizer on effectiveness data obtained from subsequent samplings at the same site. This is a particular concern in the Healthcare Personnel Handwash Test. Therefore a study was conducted comparing three different protocols based on ASTM E 1174 Standard Test Method for Evaluation of the Effectiveness of Health Care Personnel or Consumer Handwash Formulations using a 4% chlorhexidine gluconate healthcare personnel handwash formulation (Hibiclens®) as the test article. Three separate handwash protocols using 30 subjects each were performed.

HTR Study No. 01-108494-11 (Vol. III) – Industry Coalition proposed and ASTM methodology published in 2000. A chemical neutralizer was incorporated in the glove sampling fluid following the first and last wash. Effectiveness was evaluated following washes 1 and 11.

HTR Study No. 01-108495-11 (Vol. IV) – ASTM method published in 1987. This includes the elements of neutralization and inoculum similar to the methods submitted in support of NDAs. A chemical neutralizer was incorporated in the glove sampling fluid only after the last wash. Effectiveness was evaluated following washes 1,3,7, and 10.

HTR Study No. 01-108496-11 (Vol. V) – FDA 1994 proposed monograph method (59 FR 31401, proposed section 333.470, Testing of health-care antiseptic drug products). A chemical neutralizer was incorporated in the glove sampling fluid only after the last wash. Effectiveness was evaluated following washes 1 and 10.

In discussions regarding the need for neutralizers in every sampling fluid, it has been suggested that determination and quantification of the presence of the antimicrobial ingredient in the sampling fluid would also be useful in understanding the importance of the addition of neutralizer to insure accurate results. Consequently, the amount of chlorhexidine gluconate in the first sampling fluid was determined for three randomly selected subjects in each test protocol.

Appendix II in Volumes III, IV, and V describe each test protocol. Efficacy results are presented in Table 1, and the results of neutralization efficiency for each of the studies are shown in Table 2.

TABLE 1
Efficacy Results

			Inoculum			Wash Procedure				Log_{10} Reduction	
Study Number/Test Method	**Test Subjects**	**Baseline (log_{10}/hand)**	**Vol[a]**	**Rub Time**	**Dry Time**	**Wash Vol**	**Wash Time**	**Rinse Time**	**Massage Time**	**1st Wash**	**Final Wash (10th or 11th)**
HTR 01-108494 Industry Proposed, ASTM current method (E1174-00)	30	9.2172	4.5 mL	45 s	1 m	5 mL	15 s	30 s	1 m	2.4225	3.4453
HTR 01-108495 [b] NDA and former ASTM method (E1174-87)	30	9.2087	4.5 mL	45 s	1 m	5 mL	15 s	30 s	1 m	2.4622	3.7937
HTR 01-108496 Proposed monograph method	30	6.4942	5 mL	45 s	1 m	5 mL	15 s	30 s	1 m	-0.0073	1.1092

[a]Inoculum volumes in HRT 01-108494 and HTR 01-108495 were applied as 3 separate 1.5 mL aliquots.
[b]Log_{10} reduction following 7th wash 3.8906.

Data presented has been extracted from Appendix VI of reports in Volumes III, IV, and V.

TABLE 2
Neutralization Efficiency Results

		Immediate Glove Neutralization		Delayed Diluent Neutralization	
Study Number/Test Method	**Avg ppm CHG present in recovery fluid**	**Organisms Recovered (viability) Time 0**	**Organisms Recovered (viability) Time 30 min**	**Organisms Recovered (viability) Time 0**	**Organisms Recovered (viability) Time 30 min**
HTR 01-108494 Industry Proposed, ASTM current method (E1174-00)	48	90%	118%	na	na
HTR 01-108495 NDA and former ASTM method (E1174-87)	22	95%	104%	22%	4%
HTR 01-108496 Proposed monograph method	43	100%	147%	28%	<1%

Neutralizations calculated following final (10^{th} or 11^{th} wash) for all protocols. See Appendix V of reports in Volumes III, IV, and V for details.

Chlorhexidine gluconate analysis calculated following single wash.

This study supports the following conclusions:

- Incorporation of neutralizer in the sampling fluid after the first wash does not adversely affect the ability to determine a cumulative effect.
- Lack of neutralization in the sampling fluids results in an overexpression of efficacy.
- Without the inclusion of a neutralizer in the sampling fluid, organism viability decreases over time due to continued antimicrobial activity.
- Chlorhexidine gluconate is extracted by the sampling fluid - both with and without neutralizer - at levels higher than MIC values.
- The 1994 TFM test methodology is inappropriate for these products.

Incorporation of neutralizer in the sampling fluid after the first wash does not adversely affect the ability to determine a cumulative effect.

As proposed in the September 29,1999 briefing document, efficacy should be determined following a single hand wash procedure (immediately after product use), with an option for similar sampling after a total of 10 hand wash procedures to demonstrate cumulative microbial reduction from multiple washes.

It is important to assure that any proposed neutralizing method does not interfere with the assessment of other attributes that may need to be measured, such as persistence or cumulative effect.

In study HTR 01-108494 neutralizer was incorporated in the sampling fluid following both the first and last wash. As seen in Table 1, the $\log_{10}$ reductions achieved by the test material (Hibiclens®) following both samplings are within the historical range (see Table 3, page 16), but are lower than HTR 01-108495 which utilized neutralizer in the last wash only.

Lack of neutralization in the sampling fluids results in an overexpression of efficacy.

As seen in Table 1, in study HTR 01-108495 where neutralizer was present only following the final wash, the $\log_{10}$ reduction following the seventh wash (no neutralizer) is greater than the reduction seen following the tenth wash (neutralizer). This indicates that there is an overexpression of efficacy in this method when neutralizer is not included in the sampling fluid, i.e. at all time points prior to the tenth wash. This conclusion is confirmed by the data provided on organism recovery from the different fluids (Table 2).

Without the inclusion of a neutralizer in the sampling fluid, organism viability decreases over time due to continued antimicrobial activity.

Data are presented in Table 2 showing the organism viability in the sampling fluid following the tenth wash for all three protocols. Data on organism viability following the first wash are additionally presented in Volumes III, IV, and V as part of the complete protocol report for each study, but since neutralizer was only included for this wash in one protocol, no comparison can be made.

Sampling fluid without neutralizer does not quench the activity of the test article (time 0 vs. time 30 minutes) as demonstrated by the significant number of organisms recovered at time 0 and the fewer number of organisms recovered after 30 minutes at room temperature. The longer the test organisms remained in the sampling fluid (containing the test article, in this case chlorhexidine gluconate) without neutralizer the lower the percent recovery of organisms. Thus efficacy at the time of sampling may appear to be exaggerated if any delay occurs in processing the samples. By having neutralization occur in the glove following both wash procedures, the activity at the prescribed test treatment time is accurately reflected in the results. When neutralizer is not present in the glove sampling fluid, effective neutralization will not occur until further dilution of sampling fluid takes place for dilution and plating of recovered organisms, if then.

Because antimicrobials differ in their ability to be effectively neutralized by dilution e.g., alcohol being relatively easy and chlorhexidine relatively difficult, valid comparisons of their activity in a standardized test will be compromised unless the error introduced by ineffective neutralization is minimized during sampling.

Chlorhexidine gluconate is extracted by the sampling fluid - both with and without neutralizer - at levels higher than MIC values.

An analysis of the sampling fluids with and without neutralizer for chlorhexidine gluconate showed a range of from 19 to 69 ppm chlorhexidine gluconate present following a single wash. These values are significant in that they span the range of reported MIC values for the test organism. Denton (2000) reports a mean MIC of 30 ppm for 10 isolates of *Serratia marcescens* and a range of 16-64 ppm. In Table 2 it is shown that this strain is inhibited by the level of chlorhexidine present in the sample fluid, as fewer organisms are isolated from that fluid at 30 minutes as compared to time 0 if neutralizer is not incorporated into the fluid. Without neutralization, the activity of chlorhexidine gluconate continues in the sampling fluid, thus causing the appearance of increased efficacy.

The 1994 TFM test methodology is inappropriate for these products.

The surrogate endpoint efficacy data obtained from using the proposed 1994 Monograph method (HTR 01-108496, in Vol. V) illustrates a severe flaw in the protocol: determination of the organism baseline is done following both organism contamination and a cleansing wash, rather than determining the baseline after hand contamination

but prior to a wash. This results in a significant reduction of the marker organisms on the skin prior to the first test wash, resulting in an apparent lack of efficacy. This modification of the method is inappropriate as it lowers the initial inoculum to levels that are difficult to reproduce and increases the variation within the test. It is analogous to both the surgical scrub and patient pre-operative skin preparation methods where the level of bacteria on the skin at baseline significantly impacts the resulting measurement of efficacy. In all cases, if the numbers of bacteria on the skin are high, the potential efficacy of the product that can be measured is also higher. If the numbers of contaminating bacteria are low, the absolute reduction in numbers will be lower even though the reduction of bacteria by product use may be as efficient as when the contaminating bacteria are high.

b. Literature Review

In our Citizen Petition submitted to the Agency on August 6, 2001, the results of an exhaustive literature search were presented on the surrogate endpoint efficacy of healthcare personnel handwashes, surgical scrubs, and pre-operative skin preparations. The tables from Appendices D and E of that submission are referenced in this discussion and appended as Vol. 1, Tab 8 in this Citizen Petition.

The following demonstrates the importance of incorporating neutralizers in all sampling fluids during the conduct of the Standard Test Methods for Evaluation of the Effectiveness of Health Care Personnel or Consumer Handwash Formulations (ASTM E 1174), Surgical Hand Scrub Formulations (ASTM E1115), and a Pre-operative Skin Preparation (ASTM E1173).

Chlorhexidine gluconate, povidone-iodine, PCMX and triclosan are all substantive active ingredients that can be formulated for use as Healthcare Personnel or Consumer Handwashes. While chlorhexidine gluconate is not an active ingredient under consideration for this monograph, it is an NDA-approved OTC drug widely used as a Healthcare Personnel handwash. Therefore it should meet the performance criteria proposed for products regulated under this monograph. Only chlorhexidine gluconate and povidone iodine are discussed below as the number of studies conducted on these ingredients is far greater than the other active ingredients.

ASTM E 1174 Healthcare Personnel Handwash Formulations

For Healthcare Personnel Handwash Formulations, only 4% chlorhexidine gluconate formulations were reviewed for this analysis. All other active ingredient formulations had too few examples for meaningful interpretation. Using the data in Appendix D Table 1 of the August 6, 2001 submission (Vol. 1, Tab 8), the mean and median were determined for 4% chlorhexidine gluconate where it was known when neutralizers were added in the procedures, i.e. when neutralizers were incorporated only in the dilution and plating medium, or when they were incorporated in the sampling fluid placed in the glove as well as in subsequent dilution and plating steps.

Table 3
Healthcare Personnel Handwash Formulations

Active	Neutralized	Log_{10} Reduction Wash 1			Log_{10} Reduction Wash 10		
		Range	Mean	Median	Range	Mean	Median
4% Chlor-hexidine Gluconate	Media only	1.63 - 3.61	2.13 (N=9)	2.05	3.12 – 4.15	3.60 (N=8)	3.30
	Glove (Wash 10) and Media	0.28 – 3.3	1.93 (N=7)	1.97	2.77- 2.93	2.83 (N=4)	2.80

N = number of examples

As can be seen in Table 3, the inclusion of neutralizers in the glove sampling medium results in a reduction in the mean and median after the tenth wash. The effect is small after a single wash as, in most cases, neutralizer was added to the stripping fluid only after the tenth wash (this follows the 1987 and 1994 versions of the ASTM method). However, a pronounced reduction is seen when the comparison is made at the tenth wash. These data indicate that as more chlorhexidine is deposited on the hands after repeated washing, a greater amount of chlorhexidine can be removed from the skin on sampling. Without immediate neutralization in the glove sampling fluid, an overestimation of the efficacy of the formulation will result.

The effect of neutralizer can also be seen where a sample was taken after wash 7 and the bacterial reduction calculated. In all 8 examples where neutralizers were added only to the diluting media, a greater reduction was seen at wash 10 when compared to wash 7 (see Vol. I, Tab 8, Appendix D, Table 4). However, in the three examples where neutralizers were added to the glove medium at wash 10, but not added to the sampling fluid at wash 7, the reduction seen at wash 7 was greater than that seen at wash 10.

ASTM E1115 Surgical Hand Scrub Formulations

For Surgical Hand Scrub Formulations, 4% chlorhexidine gluconate and 7.5% povidone-iodine formulations were reviewed for analysis. All other active ingredient formulations had too few examples for meaningful interpretation.

Using the data in Appendix D, Tables 4 and 6 of Vol. 1, Tab 8, the mean and median were determined for 4% chlorhexidine gluconate and 7.5% povidone-iodine formulations, respectively. Only examples where it was known when neutralizers were added in the procedures were used in this analysis, i.e. when neutralizers were incorporated only in the dilution and plating medium, or when they were incorporated in the sampling fluid placed in the glove as well as in subsequent dilution and plating steps.

Table 4
Surgical Hand Scrub Formulations

Active	Neutralized	Log_{10} Reduction Day 1			Log_{10} Reduction Day 5		
		Range	Mean	Median	Range	Mean	Median
4% Chlor-hexidine Gluconate	Media only	0 – 3.64	2.31 (N=16)	2.345	1.33 – 4.18	3.41 (N=13)	3.59
	Glove and Media	-0.127 – 4.8	0.96 (N=28)	0.86	0.73 – 3.79	2.22 (N=6)	1.975
7.5% Povidone-Iodine	Media only	1.0-2.24	1.75 (N=6)	1.865	1.0-3.51	2.10 (N=6)	1.88
	Glove and Media	0.21-1.158	0.847 (N=5)	0.987	1.57	1.57 (N=1)	1.57

N = number of examples

As seen in Table 4, the mean and median values are substantially reduced if neutralizer is added to the sampling fluid in the glove. This effect is seen for both chlorhexidine gluconate and povidone-iodine at both sampling points. This indicates that as more chlorhexidine or povidone-iodine is deposited on the hands after repeated washing, the greater the amount of antibacterial ingredient that can be removed from the skin on sampling. Without neutralization immediately in the glove sampling fluid, an overestimation of the efficacy of the formulation can be made.

ASTM E1173 Pre-operative Skin Preparation

For Pre-operative Skin Preparations, only 4% chlorhexidine gluconate formulations were reviewed for analysis. All other active ingredient formulations had too few examples for meaningful interpretation.

Using the data in Appendix D, Table 7 of Vol. 1, Tab 8, the mean and median were determined for 4% chlorhexidine gluconate where it was known where neutralizers were added in the procedures, i.e. when neutralizers were incorporated only in the dilution and plating medium, or when they were incorporated in the sampling fluid placed in the cup as well as in subsequent dilution and plating steps.

Table 5
Pre-operative Skin Preparations

Active	Neutralized	Log_{10} Reduction Abdomen			Log_{10} Reduction Groin		
		Range	Mean	Median	Range	Mean	Median
4% Chlor-hexidine Gluconate	Media only or Unknown	2.53-4.95	3.32 (N=5)	3.05	3.47 - 4.04	3.68 (N=5)	3.64
	Cup and Media	1.81-1.98	1.90 (N=2)	1.90	2.13 - 4.31	3.42 (N=3)	3.81

N = number of examples

As seen in Table 5, the mean and median log_{10} reduction values at the abdomen site are substantially reduced if neutralizer is added to the sampling fluid in the cup. At the groin site, the reduction is seen in the mean value when neutralizer is added to the sampling cup; however, a similar reduction is not seen in the median value. In general there are a fairly small number of studies, which may have an effect on the interpretation of these data. However, the trends support the proposal for incorporation of neutralizer in the sampling fluid as a prudent and reasonable means of preventing the overestimation of the efficacy of a formulation.

Summary

Review of the data available in the literature using methods based on the proposed ASTM efficacy methods, as well as new data using the Healthcare Personnel Hand Wash method, clearly demonstrates the need for the incorporation of neutralizers in the sampling fluid at all time points. Comparison of three handwash protocols with a recognized effective topical antimicrobial product corroborates what is recognized in the scientific literature, namely that immediate and complete neutralization of the active ingredient is essential at all test time points. Incorporation of the neutralizer at the recommended time points will not interfere with the ability to evaluate other product attributes, including persistence or cumulative effect. In fact, it will result in an accurate assessment of product efficacy. The Industry Coalition urges FDA to revise the 1994 TFM test methodology to incorporate these critical recommendations regarding neutralization.